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Ultrasonik ve Ultrasonik Sınır Altındaki Farklı Ses Frekanslarının *Culex pipiens* (L.)'in (Diptera: Culicidae) Larvaları Üzerine Etkilerinin Değerlendirilmesi

Halil OZKURT[1] Hakan KAVUR[1*]

ÖZET: Vektör eklembacaklılar olarak bilinen sivrisinekler, insanlığa çeşitli hastalıkları bulaştırırlar. Dünya çapında, sivrisineklere karşı uygulanan mücadele programları, mikroorganizmaları, kimyasalları, doğal düşmanlarını, bazı fiziksel bariyerler gibi farklı ajanlardan oluşmaktadır. Çalışmamızda, ultrasonik ses seviyenin üstündeki ve altındaki değişen ses frekanslarının *Culex pipiens* larvalarına olan etkilerini belirlemeye çalıştık. Toplamda sekiz saat süren deneylerimizde, 3 farklı düzeyde (10.8 kHz, 20.0 kHz ve 24.5 kHz), 3 tekrarda, ses frekansı üreten piezo transdüktörlü modifiye kaplarda bulunan 720 adet sivrisinek larvası kullanılmıştır. Kontrol grubu olarak da aynı sayıda larva kullanılmıştır. *Culex pipiens*'in ikinci evre larvalarının, kullanılan frekans seviyelerine daha duyarlı olduğu bulunmuştur. Ayrıca larva mortalite açısından en etkili ses frekansı, 129 ölü larvanın gözlemlendiği, 10.8 kHz olarak tespit edilmiştir. Kullanılan üç ayrı frekans için larvalar üzerinde gözlemlenen mortal etkinin, değişen düzeylerde uygulanan ses frekansları ile doğrudan ilişkili olmadığı, larvaların her birinin akustik rezonans sınırları ile ilgili olduğu tarafımızca tespit edilmiştir.

Anahtar Kelimeler: Sivrisinek, *Culex pipiens*, Ses Frekansı, Ultrasonik, dB, Rezonans

Evaluation of the Effects of Different Ultrasonic and Under Ultrasonic Limits Sound Frequencies on the Larvae of *Culex pipiens* (L.) (Diptera: Culicidae)

ABSTRACT: Known as vector arthropods, mosquitoes transmit several types of diseases to human. Applied vector programmes worldwide against mosquitoes have different agents such as microorganisms, chemicals, natural enemies, physical barriers, etc. We aimed to determine the effects of varying sound frequencies above and below ultrasonic level (10.8 kHz, 20.0 kHz and 24.5 kHz), on *Culex pipiens* larvae with three replicates. In total, 720 mosquito larvae located in modified container with produced three different level sound frequencies piezo transducers were used in our experiment lasting eight hours. Also, the same number of larvae were used as control groups. Second instar larvae were detected more sensitive to used frequencies level. Also, in terms of larval mortality, the most effective sound frequency was 10.8 kHz in which 129 larvae died. It was concluded that the mortal effect was not related to the change in the frequency of sound; and this effect was related to resonance of the larvae used in the study.

Keywords: Mosquitoes, *Culex pipiens*, Sound Frequencies, Ultrasonic, dB, Resonance

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INTRODUCTION

Mosquitoes are the arthropod vectors of several pathogenic agents that transmit dangerous diseases worldwide as malaria, filariasis, yellow fever, dengue fever and other many viral diseases (Alten and Caglar, 1998; Khalfia et al., 2016). They are worldwide dispersed dipterans, classified in the suborder Nematocera of the family Culicidae that comprise nearly 3500 species and subspecies in 44 genera (Wilkerson et al., 2015).

Nearly 50 species in 8 genera were identified in several years in Turkey (Caglar et al., 2003; Wiley and Liebermann, 2011; Vences et al., 2013; Wilkerson et al., 2015). Moreover, *Anopheles* (*A.*) *sacharovi* and *A. superpictus* are known as main vectors of malaria that transmit *Plasmodium* (*P.*) *vivax* and *P. falciparum* in Turkey (Kuscu et al., 2014). Invasive and endemic populations of *Aedes* (*A.*) *aegypti*, the primary vector of Zika, dengue, yellow fever, and chikungunya viruses continue to expand and signify a major threat to public health worldwide (Britch et al., 2016).

In Turkey, thanks to an effective malaria control program, in 2000, the number of cases was 11381, while lowering nine cases consisted only of relapse patients in 2010, which shows that malaria was reduced by 99%; and it was categorized as the elimination phase of the World Health Organization (Kuscu et al., 2014).

Vector control programs, including chemicals (insecticides), environmental management and biological control, aim to decrease the contact between humans and the vector. Some nonchemical alternative methods and many biological agents, such as bacteria and plant extracts are used in these vector control programs. Also, in developing countries, larval control is carried out as Integrated Vector Management (IVM) programs. The novel technologies designed to kill mosquito larvae with sound waves may provide a nonchemical alternative to treat stored puddles around homes in regions inhabited by several mosquito species (Britch et al., 2016).

Sonic pest devices are tools that emit sound in the attempt to repel, deter, or kill unwanted animals such as insects, rodents, birds and large mammals. These devices, depending on the target species, cover a wide range of the acoustic spectra from below what humans perceive (infrasonic) to above our hearing range (ultrasonic). Infrasonic is characterized as the sound below 20 kHz, whereas ultrasonic sound is defined as the sound above 18.000 Hz. Ultrasonic devices are typically marketed to target arthropod (including spiders, scorpions and insect pests) and mammal pests, while devices targeting birds operate within our normal hearing range (Aflitto and Hofstetter, 2014).

Sound is a mechanical vibration wave emitted in an environment of matter. According to physicists and clinicians, sound is the molecular distribution of the energy source in the air environment, and it can be defined as the stimulus of hearing. Sound pressure is one of the most important sound magnitudes. Sound pressure is the changes of air pressure over a certain period of time during sound propagation (Ozkurt and Altuntas, 2018).

Sound pressure $P(t)$ is a size that varies by time and cannot be directly characterized as a magnitude by vibrations in the sinus form. Effective sound pressure "P" in simplified form is more practical. The average value from the varying sound pressure during the time of observed T is given by Equation 1 as follows;

$$P=\sqrt{\frac{1}{T}\int P^2(t)dt} \qquad (1)$$

Basic sound pressure is the sound pressure at the hearing limit of 1000 Hz, which is considered to be 2.10^{-5} Pa. The level of sound intensity (L_I) is the logarithm of the ratio of a physical magnitude to a given basic value in Equation 2. Another meaning of dB is the perceived volume or noise level unit.

The basic sound level I_0 for the volume level, hearing limit at 1000 Hz;

$$L_I = 10.\log \frac{I}{I_0} \qquad (2)$$

In Equation 3, the intensity is proportional to the square of the sound pressure $(I \sim p^2)$ and (L_p);

$$L_p = 10.\log \frac{p^2}{p_0^2} = 20.\log \frac{p}{p_0} \qquad (3)$$

The basic sound pressure here is (p_0); the sound pressure at the hearing limit of 1000 Hz is accepted as 2.10^{-5} Pa (Cetinkaya, 2010).

The amount of pressure changes in unit time is called the sound frequency and is measured as Hertz (Hz). Sound waves (Figure 1 and Figure 2) are in the form of a sine wave. The distance between two peaks is called "the wavelength"; and the number of wave peaks observed in a second is called frequency. In other words, the frequency of a wave depends on how often the particles in the medium vibrate as the wave passes through the air or another medium. Frequency is calculated by measuring reverse and forward vibrations depending on time. The number of vibrations per second is specifically expressed in the Hertz unit (1 Hertz = 1 cycle / second) (Isci, 2006).

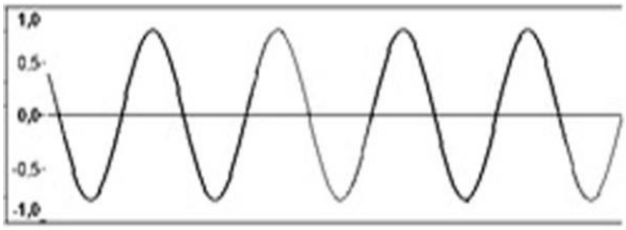

Figure 1. Low-frequency sinus wave

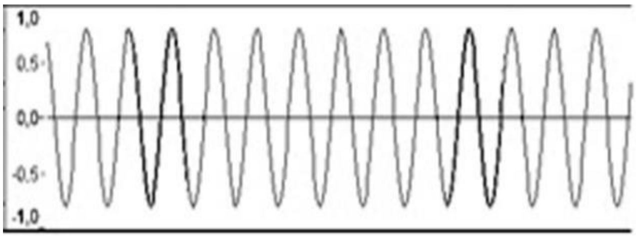

Figure 2. High-frequency sinus wave.

When the number of vibrations per second is more than 20000 (20 kHz above), this is called "ultrasonic sound". In some sources, this vibration number limit is indicated between 16000 - 20000 (16 - 20 kHz). Ultrasonic sound, used in daily life and technology, cannot be heard by the human ear (Isci, 2006).

The aim of this study was to better understand ultrasonic sound effects on *Culex pipiens* larvae and determine the most effective ultrasonic sound frequency range.

MATERIAL AND METHOD

Providing the Mosquitoes Larvae

We used *Culex pipiens* first (L_1), second (L_2), third (L_3) and fourth (L_4) instars larvae, provided from Entomology Laboratory in Cukurova University, Medical School, Department of Medical Biology in Adana.

Each frequencies experiment was conducted in two containers (control and trial container) with three replicates and totally 480 mosquito larvae [240 Control (60 L_1, 60 L_2, 60 L_3 and 60 L_4)]. Each trial containers had 80 (20 L_1, 20 L_2, 20 L_3 and 20 L_4) larvae; and the amount of the water ranged between 100 and 150 ml.

The Experimental Setup

In our experiment, adjustable frequency oscillator (Signal Generator), which can generate frequency ranges between 5 kHz and 35 kHz including ultrasonic limit and above values, was used. Also, a 400-Watt amplifier, which can adjust and apply various dB values, a frequency meter to measure the obtained frequencies in addition to the sound level meter (Noise Measurement Device), and a modified container (Figure 3), which had all instars larvae and two simple piezo transducers were used.

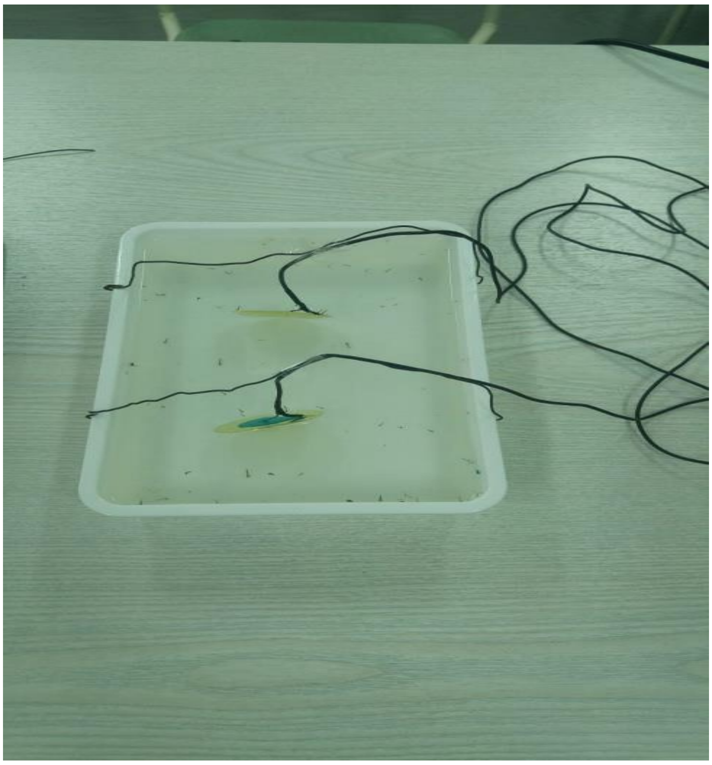

Figure 3. Piezo transducer modified container.

Mechanical energy is converted into electrical energy or vice versa by using piezoelectric feature. It is a property of some crystals and ceramics that produce electricity as a result of mechanical compression and mechanical vibration when applied electricity. In this experiment, we used square wave electric energy at certain frequencies to piezo disc material (it is possible to consider it as an electronic circuit element), which (Figure 4) was allowed to vibrate in water. As the frequency values became higher, the number of the vibrations increased in the water.

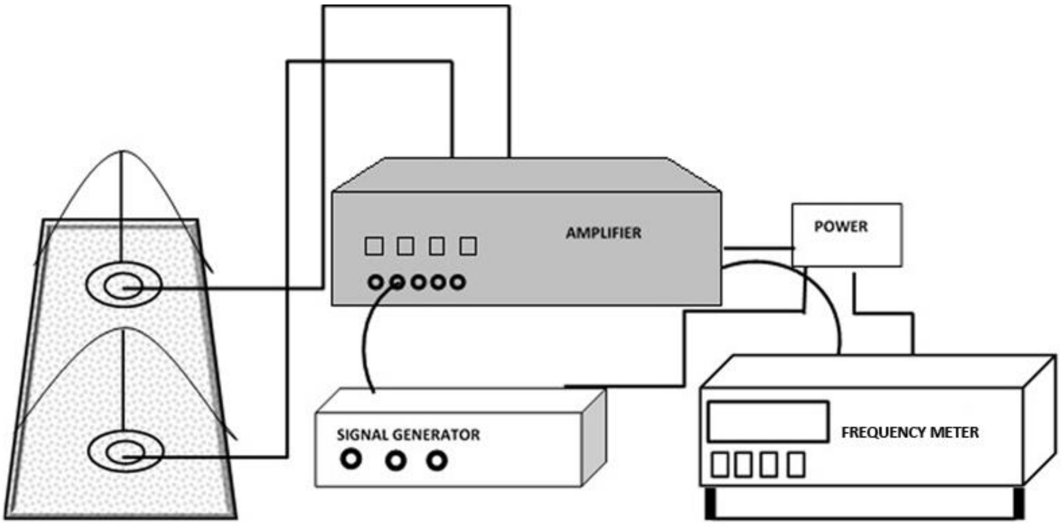

Figure 4. Experimental setup.

Trials

Larvae were exposed to three different ultrasonic sound frequencies (10.8 kHz, 20.0 kHz and 24.5 kHz) in three trials lasting eight hours, with three replicates to determine the most effective frequency range. All replicates were carried on at the same time (Okorie et al., 2015).

Before trials, we measured the air temperature, moisture and sound level inside the water with the decibel meter (Lutron; Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan) (Figure 5); then we placed the piezo transducer inside the trial container. To determine mosquito larval Total Mortality (TM) in each experiment and frequencies, 240 larvae were released into the container and dead larvae were counted in 1st, 2nd, 4th and 8th hours after the exposure.

Figure 5. Decibel meter

Statistical Analysis

All data were analysed using PASW 18.0 software (PASW; SPSS Hong Kong Headquarters, Quarry Bay, Hong Kong) with an analysis of variance (ANOVA). The means were compared with Tukey's Multiple Range Test.

RESULTS AND DISCUSSION

We employed three different ultrasonic frequencies to 720 (240 larvae*3) *Culex pipiens* larvae for a period of eight hours with three replicates. In total, 240 of 720 larvae (Table 1) were dead in our experiments. The most affected larval stage was determined as L_2 with 129 dead larvae of 240 that were exposed to ultrasonic sound frequencies. Also, there was no correlation between the time of ultrasonic sound frequencies and larval mortality.

The first trial was conducted with 10.8 kHz - 75 dB (including the environment and equipment noises) with three replicates, and it was determined that the most effective frequency in terms of total mortality was effective at an average of 28°C and 55.6% moisture. After the exposure, 126 of 240 larvae were dead at different hours. We observed that L_2 instars, all of whom died, were more affected than others. Also, 54 of 126 larvae (Figure 6) were dead in the first hour. In our first frequency exposure, the most larval mortality was observed in the 8th hour with 54 dead larvae

Table 1. Total larval mortality of trials.

Frequencies (kHz)/Sound Pressure (dB)	**L_1**	**L_2**	**L_3**	**L_4**	**Total Larvae/Mortality**	**%**
0/0 (Control)	**0**	**0**	**0**	**0**	**240/0**	**0**
10.8/75	36	60	21	9	240/126	52.5
20.0/70	30	48	0	9	240/87	36.25
24.5/66	3	21	3	0	240/27	11.25
TOTAL	**69**	**129**	**24**	**18**	**720/240**	**100**

Figure 6. Larval mortality of *Culex pipiens*, exposed to ultrasonic frequency at 10.8 kHz, in eight hours period.

We tested the mortal effects of 20.0 kHz-70 dB (including the environment and equipment noises), ultrasonic sound frequency range, on *Culex pipiens* larvae in our trial, lasting eight hours (Figure 7), at an average 28°C and 55.6% moisture and three replicates. After exposed, 87 of 240 larvae were counted as dead at different time periods. The most sensitive larval stage was L_2 with 48 dead larvae. The highest larval mortality was observed in the 8th hour period with 33 dead larvae in the second frequency trial.

Figure 7. Larval mortality of *Culex pipiens*, exposed to ultrasonic frequency at 20.0 kHz, in eight hours period.

The last ultrasonic sound frequency was 24.5 kHz-66 dB (Figure 8), which was determined to be the most ineffective in terms of larval mortality (including the environment and equipment noises) in our study at average 28°C and 55.6% moisture. We counted only 27 dead larvae, after 8 hours exposure. Among the larval stage, L_2 was found to be most affected stage with 21 dead larvae. As in other frequencies, the number of the larvae that died at the 8th hour was higher than in other time periods with 15 dead individuals.

Comparing the three frequencies employed in our trials, it was found that there was a statistically significant relation (Table 2) between the 10.8 kHz and 24.5 kHz mortality (p<0.05).

Figure 8. Larval mortality of *Culex pipiens*, exposed to ultrasonic frequency at 24.5 kHz, in eight hours period.

Table 2. Mean percentage total mortality, %TM (SE), observed in *Culex pipiens* larvae for each exposure ultrasonic sound frequences.

Groups	N	X	SD	%TM	SE	F	P	Significant Difference
Control	16	.00	.00	.00	.00			
10.8 kHz	16	2.63	3.519	52.5	.880	4.711	**.005**	10.8 kHz-24.5 kHz
20.0 kHz	16	1.81	2.373	36.25	.593			
24.5 kHz	16	.56	1.094	11.25	.273			
Total	**64**	**1.25**	**2.377**	**33.33**	**.297**			

Ultrasonic modified mosquito repellents are used alternatively instead of chemical agents, which kill nontarget organisms, cause insecticide resistance problems, affect human health and pollute the environment worldwide. They can suppress the population of vector arthropods such as mosquitoes, sand flies, cockroach, etc.

Ultrasound technologies have been promoted as an effective means of minimizing pest organisms in ponds and lakes, but little is known about the effects of ultrasound on non-target organisms (fish, frog, dog etc.,) or ecosystem processes when implemented on a large scale in complex natural systems (Aflitto and Hofstetter, 2014; LaLiberte and Haber, 2014).

In our study, we tested the effect of sound waves at different frequencies including ultrasonic level on mosquito larvae in eight hours' period. It was determined that he mortal effect varied depending on the high frequency values (including ultrasonic levels) with the effect of dB.

Although the dB value seems to be directly proportional to the mortal effect, the vibrations of the sound waves generated at different high frequencies cause changeable mortal effects on mosquito larvae (Table 1).

The difference in this mortal effect is thought to be related to the limits of resonance of mosquito larvae, interspecies or instar variation and using piezo transducers, which have different features (Britch et al., 2016; Ayannusi et al., 2018). All structures especially mosquito larvae, have different resonance. Since their tissues are sensitive, simply matching resonance of them causes acute trauma and embolism resulting in death of the mosquito larvae (Nyberg and Nyberg, 1981). For his reason, we found a relationship between under ultrasonic (10.8 kHz) and ultrasonic level (24.5 kHz) frequencies (Table 2). This relationship can be explained as both of them are out of the resonance limit. Also, according to our results, L_2 larvae are the most sensitive group in all used frequencies, considering the change of effects in different resonances.

Many researchers have examined the larvicidal activity of some stronger devices, which killed mosquito larvae in a short time at ultrasonic level. Our experimental setup, including the effects of vibrations generated by two simple piezo transducers with varying dB values in the water, is absolutely different ultrasonic bath; and larvicide devices have very high energy density (Khalfia et al., 2016; Britch et al., 2016).

Different from the studies on mosquito larvae, the mosquito biting rates for five sound frequencies (ranging from 9.6 kHz to 18.2 kHz) initially demonstrated a significant increase (ranging from around 20% to 50%), which decreased from 8.3% to 25.1% when the repellers were turned off. The biting rate significantly increased at 11.8 kHz (33.7%) when the device was turned on again (Andrade and Cabrini, 2010).

CONCLUSION

In conclusion, independent from the dB value, it is thought that the increasing vibrations caused by high frequencies at the ultrasonic level approaching the resonance limits are one of the premier factors affecting the mortality of mosquitoes. In addition, we think that the most important outcome of the study is that the increase and decrease in the sound frequency is not directly proportional to the mortal effect; however, the frequency values approaching to the resonance value of each mosquito larvae due to change the mortal effect.

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